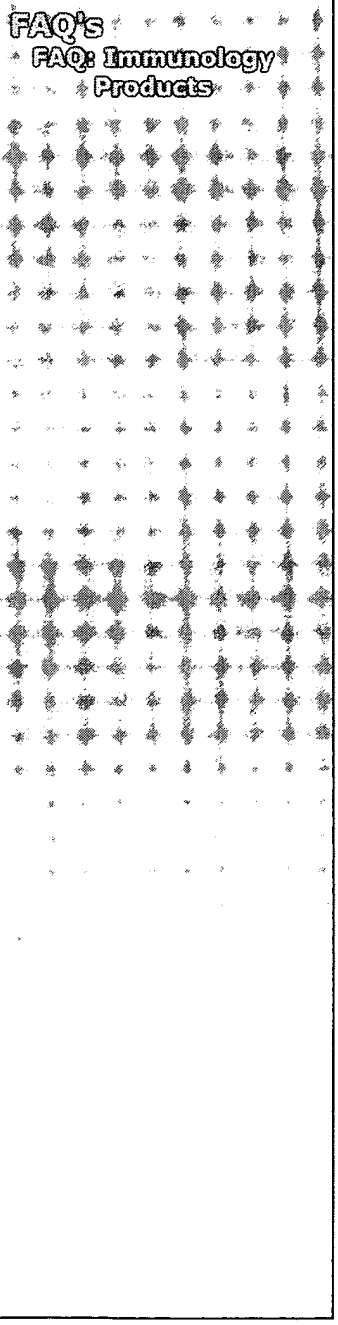

FAQ: Immunology Products

- What is the difference between PolySorp, MaxiSorp, and MiniSorp surfaces?
- What is the maximum binding capacity for proteins on the MaxiSorp surface and the PolySorp surface?
- Which 96-well Nunc-Immuno Plates or Modules are appropriate for which application?
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- What is the method for binding streptavidin followed by an addition of a biotinylated antibody using Nunc-Immuno Plates with MaxiSorp surface?
- When performing ELISA using Nunc-Immuno MaxiSorp Plates, what are some recommendations for reducing high background readings and non-specific binding?
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- What is the TSP (Transferable Solid Phase) and its advantages?
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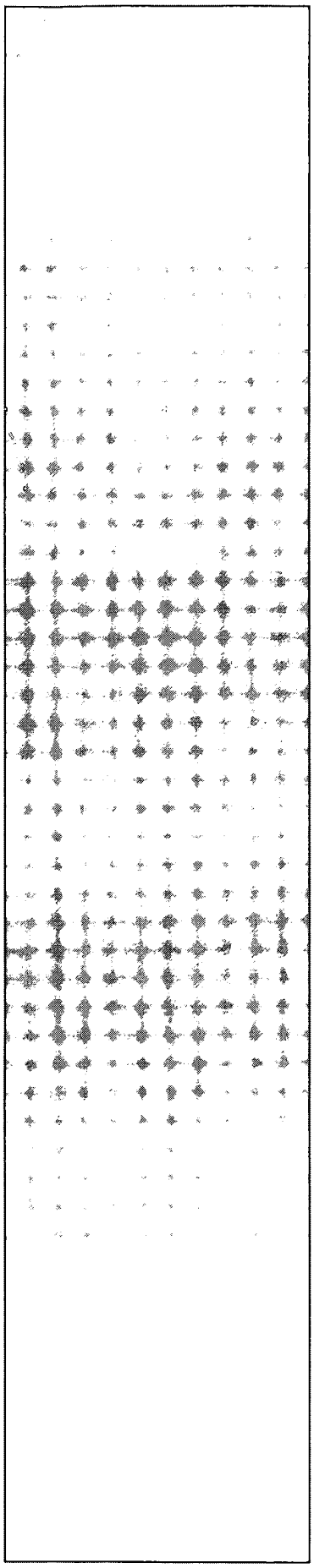
- What is the principle behind Elisa spot?
- What length of peptide is ideal for binding to the MaxiSorp surface and what are the detection limitations?

What is the difference between PolySorp, MaxiSorp, and MiniSorp surfaces?

MaxiSorp, PolySorp and MiniSorp surfaces were developed for immunology assays. The MaxiSorp surface is a modified, highly charged polystyrene surface with high affinity to molecules with polar or hydrophilic groups. The surface has a high binding capacity for proteins, including globular antibodies in proper orientation. Thus, it offers very high sensitivity in double antibody "sandwich" tests. The PolySorp surface is more hydrophobic than the MaxiSorp surface. It has high affinity to molecules of



a more hydrophobic character. This surface is particularly suited to non-protein antigens including virus antigens. The MiniSorp surface is a polyethylene surface with very low affinity to molecules of any type. This type of surface is ideal for the liquid phase immuno techniques. The MiniSorp surface is only available in a tube format, while the MaxiSorp and PolySorp surfaces are offered in both 96-well plate and module formats.

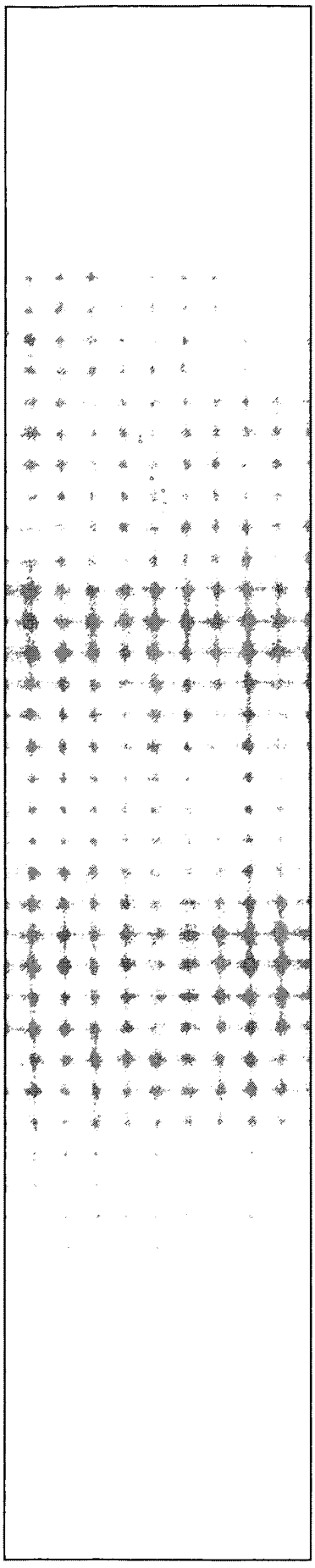
What is the maximum binding capacity for proteins on the MaxiSorp surface and the PolySorp surface?

Molecules bind to the PolySorp and MaxiSorp surfaces through passive adsorption. Using IgG as a reference molecule and knowing that it is a globular molecule, theoretical calculations indicate that the maximum binding for the MaxiSorp surface, in monolayer, is 650 ng/cm^2. For the PolySorp surface, the binding capacity is 220 ng/cm^2. A detailed discussion of the principles and calculations is presented in Nunc Bulletin No. 6. See Nunc Bulletin No. 6.

Which 96-well Nunc-Immuno Plates or Modules are appropriate for which application?

The following list offers a brief description of the features of Nunc products and their specific applications.

- Nunc-Immuno Plates, MaxiSorp surface - These plates are designed for solid phase immuno assays and have a polystyrene surface with high affinity for polar groups and hydrophilic molecules. These 96 well plates are available with flat (F), round (U) or (C) bottom-well designs.
- Nunc-Immuno Plates, PolySorp surface - These plates have a polystyrene surface which adsorps less polar molecules compared to the MaxiSorp surface and has a high affinity for hydrophobic groups. These 96 well plates are available with flat (F), round (U) or (C) bottom-well designs.
- Nunc-Immuno Modules - These modules are designed for solid-phase immuno assays. The modules are available in 8-, 12- or 16-well formats with (F), (U) and (C) bottom-well and 8-well BreakApart with (C) bottom-well designs. These different formats allow one to choose the style which is appropriate for their assay design. BreakApart Modules and LockWell Modules can be used for radioimmunoassays.
- Nunc-Immuno StarWell Modules - The 8-well modules feature eight fins on the inner wall of the (C) bottom wells. This design increases the surface area by 50%. The increase in surface area allows more molecules to be immobilized, increasing assay signal. The fin configuration provides shorter diffusion distance to the surface, reducing incubation times.
- LockWell Modules - Each plate consists of 1 x 8 breakable strips. These strips are assembled in a patented designed frame which locks each well into place by a spring lock. This spring lock design orientates each well at the same horizontal level allowing uniform washing and reading. The LockWell Modules are available with round (U), (C) or StarWell bottom-well designs.
- CovaLink Modules - These plates can be used to bind proteins,



peptides, DNA or carbohydrates. Covalent linkage occurs via secondary amine group. This allows a specific orientation and provides improved stability compared to passive adsorption due to chemical binding between molecules. Binding of molecules on PolySorp and MaxiSorp surfaces is obtained by passive adsorption. CovaLink is a surface grafted with secondary amino groups (>NH) which serves as a bridge for covalent coupling. The covalent binding can immobilize small molecules such as biotin or peptides which may otherwise bind weakly by physical adsorption and facilitate the recognition by detection molecule.

- FluoroNunc Modules and Plates - These plates are optimized for IFMA (Immunofluorometric Assays) or FIA (Fluorometric Immuno Assays). The transparent polystyrene plates give a low background fluorescence and are optimal where a read-through system is used. The white plates/modules provide maximum reflection of fluorescence signal while maintaining low background. The white plates/modules are often used for epifluorescence reading. Black modules reduce background fluorescence and minimize back-scatter light which is often encountered in epifluorescence.

Which surfaces are recommended for RIA (radioimmunoassay)?

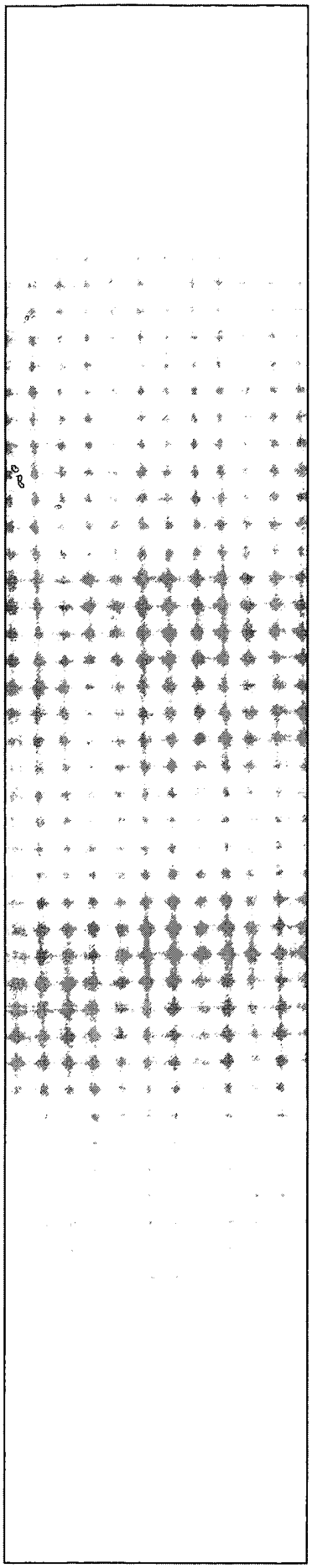
The choice of plates or modules will depend on the type of RIA you are planning to perform and specific assay conditions. Nunc provides two different surfaces for immunoassays, PolySorp and MaxiSorp. The MaxiSorp surface is highly charged and should be used if the assay requires quantitative measurement of proteins (antibodies) or molecules with polar groups. The PolySorp surface should be used if the assay consists of less polar molecules with hydrophobic characteristics.

What is the method for binding streptavidin followed by an addition of a biotinylated antibody using Nunc-Immuno Plates with MaxiSorp surface?

Streptavidin has a very low affinity to polystyrene and is most commonly immobilized using the protein-avian-biotin-capture (PABC) system. In this system, streptavidin acts as a bridge between the capture antibody and an irrelevant, biotinylated protein which is adsorbed to a solid phase. Streptavidin bound directly to plastic surfaces is impaired in both its ability to bind to biotinylated monoclonal antibody and in its functional affinity when compared to streptavidin used in the PABC system. Therefore, it is not recommended to use streptavidin hydrophobically adsorbed to solid phase to immobilize biotinylated molecules. Passive adsorption of streptavidin to Nunc MaxiSorp plates can be achieved by using conventional methods: 0.1 mg/ml, carbonate buffer (pH 9.6) with overnight incubation at room temperature. To optimize some use a buffer with a pH of 5 or 6.

When performing ELISA using Nunc-Immuno MaxiSorp Plates, what are some recommendations for reducing high background readings and non-specific binding?

Assay sensitivity depends strongly on an efficient removal of non-specific reacting molecules. High background readings and coating instability can



be eliminated by addition of a blocking step after the first coating. The excess surface is then occupied by indifferent molecules. We recommend washing three times after each coating step by using a solution of 0.15 M phosphate buffer (pH 7.2) with 0.2 M NaCl and 0.05% Tween 20. For blocking, we recommend using 0.5% BSA, 1% casein or 1% gelatin in 0.15 M phosphate buffer (pH 8.2) or carbonate buffer (pH 9.6). See Nunc Bulletins No. 7, 8, and 9.

What is the difference between certified and non-certified MaxiSorp plates and modules?

Both of these surfaces are identical. The only difference between them is that for the certified plates, a representative sample from each manufacturing lot undergoes a Binding Capacity test. This test is an ELISA-like assay used in our quality control laboratories to ensure binding capabilities. See Nunc Bulletin No. 4.

What is the stability of the secondary amine CovaLink surface with protein or DNA bound to the surface?

DNA, proteins or peptides bound to the CovaLink surface can be stored at 4°C for up to one month.

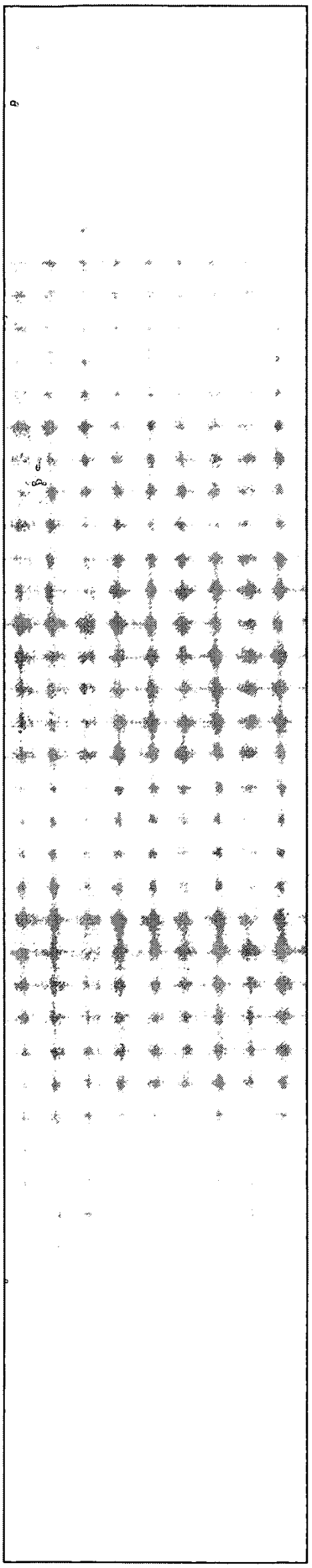
What are the features of the secondary amine CovaLink surface?

CovaLink NH Modules are surface modified optically clear polystyrene modules in strips of eight. They allow covalent binding of distinct groups of proteins, peptides, oligosaccharides and DNA. This covalent-binding feature allows orientation of the bound molecules such that the active site of the molecule is available for biochemical activity. A key feature of the CovaLink is that the polystyrene surface is grafted with secondary amino groups which serve as bridges for covalent binding. The optically clear surface allows reading of fluorescent or colorimetric signals.

What are some applications of CovaLink Modules?

1. CovaLink modules are used for immunoassays allowing an orientation of the immobilized molecule.
2. Detection of antibody levels to polysaccharide components of infectious agents. See Tech Note Vol. 2 No. 11.
3. Signal amplification of target by the use of covalently bound primer and hybridization procedures. CovaLink NH is a good product for hybridization of amplification product with covalently bound probe. Amplification is performed and hybridization of the amplified product is accomplished using CovaLink Modules (See Rasmussen et al., Clin. Chem. 40(2), pp. 200-5 (1994).

When is the use of CovaLink Modules necessary?



CovaLink is recommended for use with molecules which adsorb with difficulty to a traditional surface. Because of covalent binding very thorough washing is possible allowing orientation of the molecule and better recognition by the detection molecule.

What are some recommendations for improving binding efficiencyof proteins and DNA (oligonucleotides) using secondary amine CovaLink modules?

Use a freshly made methylimidazole and carbodiimide condensing agents for optimal covalent binding of both proteins and DNA (oligonucleotides). The binding efficiency of single stranded oligo (a 5' phosphorylated end of a single-stranded oligomer binds with a phosphoramidate bond) is about 8 - 10% with a typical 25 base oligo.

Will Nunc-Immuno plates/modules fit into an automated microtiter plate reader or washer?

Yes. All Nunc 96-well plates and frames for modules have a standard 96-well footprint, 86 x 128 mm.

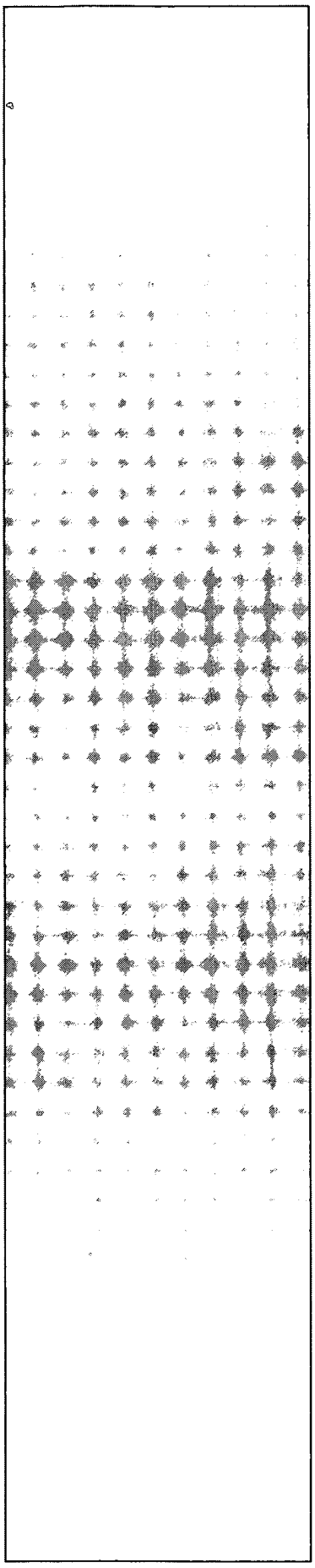
What are the advantages of one well geometry type over another? Which is best for which application?

The following list describes the geometries of wells available for Nunc Immuno-plates and modules.

- Flat bottom (F) - Allows maximum transmission of light. These plates can be read on a monochromatic reader.
- Round bottom (U) - This geometry optimizes washing and coating. These plates can be read using a dual wavelength reader.
- "C" bottom (C) - This design of the well is a combination of both flat and round bottoms. Basically, it is a flat bottomed well with curved edges at the bottom. These plates also can be read using a monochromatic reader combining optimal reading and washing.
- StarWell - These wells have a modified "C" shape geometry with eight fins strategically placed at the bottom. This feature increases surface area, allowing more molecules to become immobilized which reduces incubation times.

Does Nunc offer a manual plate washer for immunology assays, such as ELISA?

Nunc offers a manual plate washer which is compatible with 96-well plates. Nunc-Immuno Wash 8 is Cat. No. 470174 and Nunc-Immuno Wash 12 is Cat. No. 470175. Each Immuno Wash has two fittings. One fitting is connected to the wash buffer solution and the other to a vacuum line for aspiration. The wash buffer solution is dispensed manually.



Which components are included in the Nunc-Immuno Wash Tubing Kit?

The tubing kit, Cat. No. 654569, consists of tubing, Y-shaped adaptor, and three red clamps.

Is the Eight Well Strip Cap compatible with LockWell Modules?

No, due to the locking feature design of the LockWell Modules, the Eight Well Strip Cap is not compatible and will not seal the LockWell Modules.

Is the Eight Well Strip Cap compatible with all 96-well MicroWell or Immuno Plates?

The Eight Well Strip Caps were designed to provide a positive seal for flat and round bottom wells of 96 MicroWell or Nunc-Immuno plates. The Eight Well Strip Caps are not compatible with C or V bottom wells of 96 MicroWell or Nunc-Immuno Plates.

Is it possible to bind either single- or double-stranded DNA to the MaxiSorp surface?

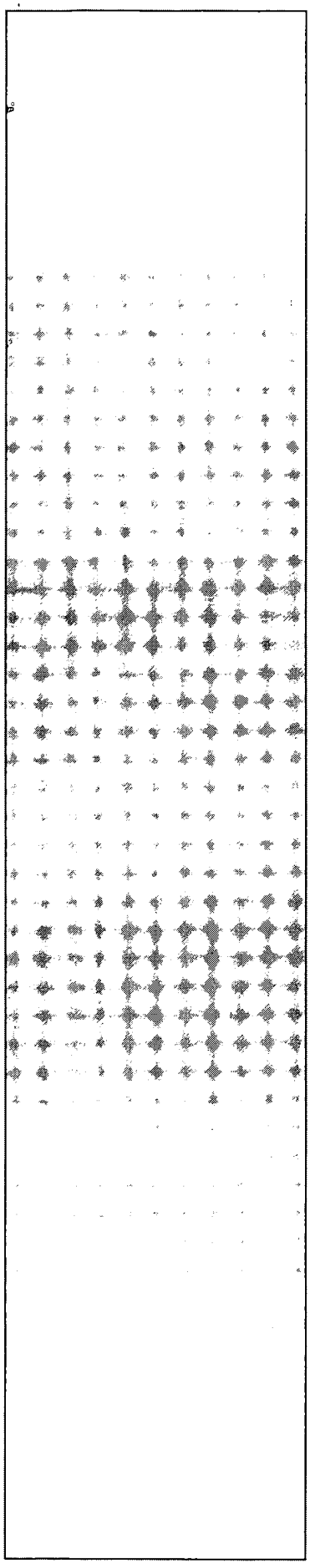
Single-stranded DNA can be adsorbed to MaxiSorp surface using approximately 10 µg ssDNA per ml PBS, pH 8.2. The stability is uncertain. Based on our experience, ssDNA immobilized on the MaxiSorp surface is so loosely bound that it is removed by stringent washing. Double-stranded DNA will not bind to the MaxiSorp surface. DNA, however, can be covalently bound to NucleoLink Strips, Cat. No. 248259.

What is the TSP (Transferable Solid Phase) and its advantages?

The TSP is a disposable 96 pin device on which solid phase reactions can be performed. It is available with a PolySorp and MaxiSorp surface. The pins are coated by submerging in analyte solution contained in a 96-well plate. Washing and reaction with succeeding antibody or streptavidin conjugates can be performed by transferring the TSP into a washing tray. For hybridoma screening, the TSP is available in a sterile version. Likewise, the TSP can be used for a simultaneous detection of two different molecules in the same solution. Advantages include: identical reaction times on all pins, no need for plate washer/dispenser, and allows for a second solid phase reaction to be conducted in a single 96-well plate.

What are some applications using the TSP (Transferable Solid Phase)?

Reactions such as the ELISA can be performed on the TSP. The pins are coated by submerging in the analyte solution contained in a 96-well plate. Washing and reaction with succeeding antibody or streptavidin conjugates can be performed by transferring the TSP into a washing tray or second 96-well plate filled with the appropriate solution. The TSP is placed into a substrate solution until color is observed and is then removed to ensure a



simultaneous start and stop to the enzymatic reaction. The TSP is available sterile for screening hybridoma cells for the production and secretion of monoclonal antibodies. For radioimmune assays, the TSP can be placed directly on X-ray film and exposed for several hours. Only the tips of the pins should be incubated with the radiolabeled reagent. The TSP can also be used with the OmniTray for performing dot blots and for replicating bacterial clones from a 96-well plate. We offer a Tech Note, Vol. 3 No. 24, that describes in situ screening of bacterial colonies using Nunc-Immuno TSP

What is the principle behind Elisa spot?

The Elisa spot technique was originally described by Sedgwick & Holt, Journal of Immunological Methods, 57, 301, 1983. The basic principle is as follows:

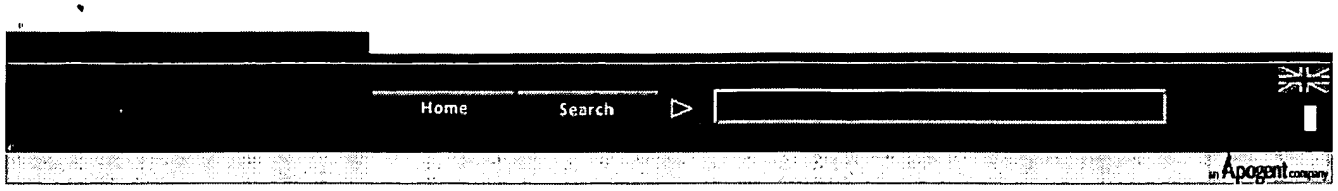
1. Coat solid phase with antigen
2. Block free sites using serum
3. Add antibody producing cells (plasma cells) from animal, e.g. mouse sensitized with the coated antigen
4. Incubate
5. Wash away cells
6. Add anti-mouse antibody conjugated with alkaline phosphatase
7. Incubate
8. Wash
9. Add substrate
10. Incubate
11. Read number of spots (converted substrate)

Elisa spot can also be used to assay products secreted from cells placed in contact with antibody coated on the solid phase.

What length of peptide is ideal for binding to the MaxiSorp surface and what are the detection limitations?

We have tested and found that a 3 amino acid peptide (Pro, Leu, Gly) cannot be detected when passively adsorbed on the MaxiSorp surface. However, this peptide can be detected when covalently immobilized using CovaLink NH Modules and CovaLink NH2 Modules and Plates. Using covalent immobilization of small peptide residues, one can obtain a better orientation of the molecule and reduced problems with antibody recognition of the peptide due to masking of the epitope.

We have discovered that a 7 amino acid peptide from the MHC Class II antigen can be detected when adsorbed on the MaxiSorp surface. We state that the detection limitation using the MaxiSorp surface is between 3 and 7 amino acid residues. One additional note is that detection is contingent upon the orientation of the peptide when immobilized. If the active site is inactivated or hidden at the site facing the solid phase, no detection signal is observed.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